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HUVEC- fibroblast co-culture model. Both 2-MeOE2MATE and 2-MeOE2bisMATE inhibited HUVEC proliferation (IC_{50}s, 0.1μM and <0.1μM respectively) to a greater degree than 2-MeOE2 (IC_{50} 1.0μM). At 0.1μM, 2-MeOE2 had little affect on tubule formation whereas 2-MeOE2MATE and 2-MeOE2bisMATE inhibited formation by 50% and 70% respectively at this concentration. To assess the efficacy of 2-MeOE2MATE and 2-MeOE2bisMATE *in vivo*, tumors derived from MDA-MB-435 breast cancer cells were inoculated into nude mice with compounds being administered at 20mg/kg/d, p.o, for a 28 day period. Both compounds significantly reduced tumor growth (2-MeOE2MATE, 34%, $p< 0.05$; 2-MeOE2bisMATE, 50%, $p<0.001$) compared with controls. Tumor volumes were monitored for a further 28 day period at which time tumor volumes in animals previously given 2-MeOE2bisMATE remained significantly smaller than those of controls (2-MeOE2bisMATE, 1.07 ± 0.15cm^3, mean ± s.e., n = 12; controls, 2.63 ± 0.28cm^3, $p<0.001$). To examine the ability of the compounds to bind to the colchicine binding site of tubulin, a mechanism by which they may act, [^{3}H] colchicine was incubated in the absence or presence of inhibitors. Both 2-MeOE2MATE and 2-MeOE2bisMATE competed for binding to the colchicine site (60 ± 2% and 55 ± 4% respectively) to a greater extent than did 2-MeOE2 (40 ± 2%). The addition of sulfamoyl moieties to 2-MeOE2 greatly enhances its potency as an anti-cancer agent and 2-MeOE2bisMATE is currently undergoing *in vivo* testing with the National Cancer Institute.

#902 2-Methoxyestradiol (2ME2) destabilizes microtubules and prevents HIF-1α nuclear accumulation and activity leading to inhibition of angiogenesis. Nicola J. Masbjeesh, Theresa La Vallee, Victor S. Pribluda, Yuefang Wang, Daniel Escuin, Hua Zhong, Jonathan W. Simons, and Paraskevi Giannakakou. *Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA, and EntreMed, Inc, Rockville, MD.*

2-Methoxyestradiol (2ME2), an endogenous metabolite of estradiol, inhibits tubulin polymerization, and has antitumor and antiangiogenic activity both *in vivo* and *in vitro*. The mechanism of 2ME2 antiangiogenic activity, however, is not known. It is well established that hypoxia-inducible factor 1 (HIF-1) promotes the expression of VEGF which plays a major role in inducing angiogenesis in solid tumors. HIF-1 is a heterodimeric protein composed of HIF-1α and HIF-1β subunits. HIF-1β is constitutively expressed, whereas HIF-1α expression is induced when cells are exposed to hypoxia. Therefore, we investigated whether the antiangiogenic effect of 2ME2 could be mediated in part through its effects on HIF-1α. Nuclear HIF-1α protein levels were decreased after treatment of PC-3 cells with 2ME2, while HIF-1β levels were not affected. These data were confirmed by confocal microscopy showing decreased HIF-1α nuclear accumulation after treatment with 2ME2 under normoxic or hypoxic conditions. The 2ME2-induced tubulin depolymerization correlated with the decrease of HIF-1α protein levels in a dose-dependent manner. To further tighten the correlation between disruption of the microtubule (MT)-cytoskeleton and inhibition of HIF-1α nuclear translocation, additional MT-targeting drugs were tested. Similar to 2ME2, disruption of the MT-cytoskeleton with taxol or vinscristine reduced HIF-1α protein levels as well as its translocation into the nucleus. In addition, tubulin sedimentation experiments revealed that HIF-1α protein preferentially co-sediments with the polymerized form of tubulin. Most importantly, treatment with 2ME2, taxol, or vincristine inhibited HIF-1 transcriptional activity *in vitro*. *In vivo* experiments using nude mice bearing human breast or glioblastoma xenografts showed a significant decrease in neovascularization after treatment with 2ME2. Herein, we propose that disruption of MT-cytoskeleton by 2ME2 inhibits HIF-1 transcriptional activity and plays an important role in the inhibition of angiogenesis mediated by this drug.

#903 Novel 2-methoxyestradiol analogs with superior activities. Tina L. Tinley, Rachel M. Leal, James W. Cessac, Pemmaraju N. Rao, and Susan L. Mooberry. *Southwest Foundation for Biomedical Research, San Antonio, TX.*

2-Methoxyestradiol (2-ME2) is an antitumor agent that appears to work via a dual mechanism, as it has both antiangiogenic activity and direct cytotoxic effects on tumor cells. Phase I and Phase II clinical trials with 2-ME2 are currently underway. The goal of this study was to evaluate the *in vitro* biological activities of 18 novel analogs of 2-ME2 for properties that may predict superior antitumor effects. The analogs were tested for activities that predict antiangiogenic activity and for direct inhibition of a variety of cancer cell types. Our results show that some of the analogs are clearly superior to 2-ME2. Inhibition of endothelial cell proliferation and inhibition of endothelial cell invasion through basement membrane materials are *in vitro* assays that are used to predict antiangiogenic activity. Specific 2-ME2 analogs exhibit greater potency with regard to inhibition of proliferation of human umbilical vein endothelial cells (HUVEC). Additionally, one analog appears to have specificity toward inhibition of endothelial cell invasion as compared to endothelial cell antiproliferative effects. It is not known if the antiangiogenic effects of 2-ME2, its direct effects on cancer cells, or a combination of both is most important for antitumor activity. Thus, the 2-ME2 analogs were also tested for direct antiproliferative effects against cancer cell lines. The results show that certain analogs are potent inhibitors of cell proliferation, with IC_{50}s in drug sensitive and multidrug resistant breast cancer cells of 0.055-4.5 μM. Some analogs are more potent than 2-ME2 as cytotoxins towards specific cancer cell lines including MCF7. One analog is an effective cytotoxin against the DU 145 prostate cell line, a line that is resistant to the cytotoxic effects of the parental compound, 2-ME2. All of the analogs retain efficacy and potency against the multidrug resistant NCI/ADR cell line. The data suggest that the analogs are not substrates for transport by P-glycoprotein and thus they should retain activity towards multidrug resistant tumors. The 2-ME2 analogs have a mechanism of action consistent with 2-ME2. The analogs cause microtubule depolymerization, the formation of abnormal mitotic spindles, and the breakdown of the nuclei into micronuclei, effects consistent with other antimitotic agents. Our data with these analogs of 2-ME2 suggest that there are differences in their specificity towards endothelial effects as well as their direct effects on tumor cells. These differences in specificity may translate into different antitumor efficacies.

#904 Tumor inhibition by anti-angiogenic TSP-1 mimetic peptides. Jack Henkin, Fortuna Haviv, Yi-Chun Wang, David Frost, Abdullah Kherzai, Frank K. Reiher, Olga V. Volpert, Susan E. Crawford, Noel P. Bouck, Steven C. Campbell, and Chand Khanna. *Abbott Laboratories, Abbott Park, IL, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Med School, Chicago, IL, and Animal Cancer Institute, Columbia, MD.*

Thrombospondin-1 (TSP-1) is a protein inhibitor of angiogenesis that blocks many functions of activated endothelial cells (EC), thereby slowing tumor growth. Its large size and multi-functionality prevent direct use in cancer therapy. We reported that a capped heptapeptide sequence from the second type-1 repeat of TSP-1, inhibits bFGF-induced EC migration, only if the Ile at position 3 was changed to Dlle. Hypothesizing that this could yield *in vivo* tumor supression, further structure-modifications led to two nonapeptides enantiomers, ABT-526 and ABT-510, that were parenterally bioavailable in the mouse, and both of which inhibited human microvascular EC chemotaxis induced by bFGF, or VEGF, and abrogated EC tube formation in fibrin gels (F. Haviv et al, 93rd AACR abstract). At 100 ng/ml they inhibited by > 90% *in vitro* proliferation of bovine capillary EC (BP10T8) but had no effect on the growth of B16 melanoma or 253J B-V bladder cancer cells. ABT-510, delivered continuously at 10 mg/kg/day, reduced by 50% the rapid flank growth (day 12) of the mouse hemangioma b.END3 in nude mice. ABT-526 treatment reduced the number and diameter of visible tumors from melanoma outgrowth in the lungs of syngeneic mice with dose-dependence from 40-200 mg/kg/day, and tumor edges from treated animals had 3-fold higher EC apoptosis. ABT-510 gave dose-dependent reduction in tumor size when given ip to nude mice in which low-TSP 253J B-V transitional cell carcinoma was implanted in the bladder wall. Treatment (27 mg/kg bid) enhanced EC apoptosis at the tumor edge by 2-fold, and decreased microvessel density by 50%. In nude mice where 1 mm3 fragments of human breast MDA-MB-435 tumor were implanted in the flank, continuous sc infusion of 0.3 mg/kg/day, or single bolus doses of 3 mg/kg/day ABT-510 gave 50% reduction in tumor growth. In a faster growing version using a direct MDA-435-LM cell inoculation into the flanks of SCID mice, a continuous infusion of ABT-510 at 30 mg/kg/day gave 36% growth inhibition at day 31. In pet dogs with naturally occurring cancers (advanced stage and/or refractory to conventional therapy; (n>75 cases) ABT-526 given as bid sc injections resulted in not only unexpected disease stabilization but also objective tumor regressions, without clinically observed toxicity in the animals. These TSP-1 mimetic peptides represent a promising approach to anti-angiogenic therapy of tumors.

#905 Targeting of bladder cancer vascular endothelium with VEGF$_{121}$/rGel fusion toxin. Daniel M. Kedar, Paul Sweeney, Samuel Huang, Badar Mian, Colin P. N. Dinney, and Micheal G. Rosenblum. *MD Anderson Cancer Center, The Departments of Urology and Bioimmunotherapy, Houston, TX.*

Development of new blood vessels is essential for the growth and metastatic spread of solid tumors. Vascular endothelial growth factor (VEGF) plays a central role in tumor neovascularization. Both VEGF and its receptors (flt-1 and KDR) are over expressed by human bladder cancer cells and tumor endothelial cells respectively. Therefore, novel therapeutic strategies, which target VEGF receptors on tumor endothelial cells, hold promise as an antiangiogenic therapeutic approach for bladder cancer. A fusion protein of VEGF121 and the plant toxin gelonin (rGel) was constructed, expressed in bacteria and purified to homogeneity. The VEGF121/rGel fusion protein demonstrated the biological activities of both VEGF and rGel components. Previous studies suggest that the KDR receptor is primarily responsible for development of VEGF121/rGel induced cytotoxicity. We evaluated the therapeutic and antiangiogenic effect of the fusion protein VEGF121/rGel against human bladder cancer xenografts growing in athymic nude mice. *In vivo* and *in vitro* studies were done with the highly metastataic 253J B-V human bladder cancer cell line. Western blot analysis for phosphorylated KDR receptor as well as cytotoxicity assay with the fusion construct were performed. 253J B-V cells were implanted orthotopically in nude mice. The mice were treated (IV every other day) with total of 20 mg/kg of VEGF121/rGel, rGel or saline. The mice were necropsied 28 days after tumor implantation. Bladder tumors were harvested, weighed and processed. Immunohistochemical studies for the KDR receptor, blood vessel endothelium (CD-31) and gelonin were also performed. Treatment with the VEGF121/rGel resulted in significant suppression of bladder tumor growth compared to controls. Western blot analysis on 253J B-V tumor cells themselves showed no detectable expression of KDR and no increased sensititvity to the construct compared to rGel itself. This ruled out a direct effect of VEGF121/rGEL on the 253J B-V cells and suggests that the *in vivo* effects appear to be solely mediated by effects on tumor neovasculature. Immunofluorescent staining with antibodies to CD-31 (blood vessel endothelium) and to rGel demonstrated dramatic co-localization of the construct on the tumor neovascul-